AFRL-ML-TY-TP-2004-4522

Screening of Fungi for Biodegradation of Volatile Organic Compounds

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	20 Apr 2004	Proceedings May 2000–May 2001

4. TITLE AND SUBTITLE Screening of Fungi for Biodegradation of Volatile Organic Compounds

5. FUNDING NUMBERS
Contract Nos: F08637-01-C-6002 and F08637-98-C-6005
PE: 63716D

5. AUTHORS Bing Qi, William M. Moe and Kerry A. Kinney

6. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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7. PERFORMING ORGANIZATION REPORT NUMBER

8. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AFRL/MLQL
139 Barnes Drive, Suite 2
Tyndall AFB, FL 32403-5323

9. SPONSORING/MONITORING AGENCY REPORT NUMBER
AFRL-ML-TY-TP-2004-4522

11. SUPPLEMENTARY NOTES
Technical monitor: Dr Joe Wander, AFRL/MLQF, 850-283-6240 [DSN 523-6240]

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Public release authorized. Distribution unlimited.

12b. DISTRIBUTION CODE
A

13. ABSTRACT *(Maximum 200 words)*
Fungi are proposed as a more-robust alternative to bacteria in biofilter treatment. In the studies described herein, five fungal species, *Exophiala lecanii-corni, Mucor rouxii* (ATCC 44260), *Phanerochaete chrysosporium* (ATCC 24725), *Cladosporium sphaerospermum* (ATCC 200384), and *Cladosporium resinae* (ATCC 34066) were used to degrade compounds commonly found in paint spray booth off-gases (benzene, *p*-xylene, methyl ethyl ketone and *n*-butyl acetate). Fungal cultures were inoculated on ceramic support media for testing at pH values ranging from 3.5 to 6.5. Fungal ability to degrade each VOC was determined by observing presence or absence of visible growth on the ceramic support media during a 30-day test period. Results indicate that *Exophiala lecanii-corni* and *Cladosporium sphaerospermu* can readily utilize. [Continued on p. ii]

14. SUBJECT TERMS	15. NUMBER OF PAGES
Biofilter; fungal degradation; VOCs; fungi; *Exophiala*; *Phanerochaete*; *Cladosporium*	16
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

NSN 7540-01-280-5500 Computer Generated STANDARD FORM 298 (Rev 2-89)
Prescribed by ANSI Std 239-18
298-102

13. ABSTRACT (CONTINUED)

n-butyl acetate, methyl ethyl ketone, benzene, and *p*-xylene as their sole carbon and energy source. *Phanerochaete chrysosporium* was able to degrade *n*-butyl acetate, methyl ethyl ketone, and benzene but not *p*-xylene under the conditions tested. *Cladosporium resinae* was able to degrade *n*-butyl acetate and methyl ethyl ketone but not benzene or *p*-xylene under the conditions tested. *Mucor rouxii* was able to use *n*-butyl acetate as a sole carbon and energy source; however, it was unable to utilize any of the other VOCs tested under the conditions imposed. Maximum growth for most of the tested fungi was observed at a *p*H value of approximately 5.0.

Screening of Fungi for Biodegradation of Volatile Organic Compounds

Paper #1005

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ABSTRACT

Many industrial practices and waste treatment operations produce gas streams containing volatile organic compounds (VOCs) that require treatment. Although biological treatment has been applied successfully to treat many waste gases, the literature contains numerous reports of diminished treatment performance under conditions of excessive drying or low pH in biofilter beds. Use of fungi in biofilter treatment systems may mitigate these effects. Recent research indicates that some fungal species are able to tolerate low pH conditions and low moisture environments. Although many fungal species have been tested for their ability to degrade non-volatile compounds, relatively few accounts of fungal degradation of VOCs have appeared in the literature to date.

In the studies described herein, five fungal species, *Exophiala lecanii-corni*, *Mucor rouxii* (ATCC 44260), *Phanerochaete chrysosporium* (ATCC 24725), *Cladosporium sphaerospermum* (ATCC 200384), and *Cladosporium resinae* (ATCC 34066) were screened for their ability to degrade compounds commonly found in paint spray booth off-gases. Fungal cultures inoculated on to ceramic support media were provided VOCs as their sole carbon and energy sources. Compounds tested included aromatic hydrocarbons (benzene, p-xylene), ketones (methyl ethyl ketone), and an organic acid (n-butyl acetate). Experiments were conducted using a variety of pH values ranging from 3.5 to 6.5. Fungal ability to degrade each VOC was determined by observing presence or absence of visible growth on the ceramic support media during a 30-day test period. Results indicate that *Exophiala lecanii-corni* and *Cladosporium sphaerospermum* can readily utilize n-butyl acetate, methyl ethyl ketone, benzene, and p-xylene as their sole carbon and energy source. *Phanerochaete chrysosporium* was able to degrade n-butyl acetate, methyl ethyl ketone, and benzene but not p-xylene under the conditions tested. *Cladosporium resinae* was able to degrade n-butyl acetate and methyl ethyl ketone but not benzene or p-xylene under the conditions tested. *Mucor rouxii* was able to use n-butyl acetate as a sole carbon and energy source; however, it was unable to utilize any of the other VOCs tested under the conditions imposed. Maximum growth for most of the tested fungi was observed at a pH value of approximately 5.0.

Results of these screening studies reveal that not only are several fungal species capable of degrading the compounds tested, they also suggest that the experimental methods described herein may be of use in conducting further screening studies for candidate microorganisms.

Keywords: Biofilter, fungal degradation, VOCs, fungi, pH

INTRODUCTION

The concerns of air pollution by volatile organic compounds (VOCs) emitted from industrial practices and waste treatment operations have prompted the development of air pollution control technologies. Among the various emerging technologies, biofiltration is considered a promising option for the treatment of air streams contaminated by VOCs at low concentrations. Benefits of this technology including relatively low operating costs, low energy requirements, and the avoidance of cross media transfer of pollutants[1].

Currently, most biofilter applications utilize undefined mixed cultures comprised primarily of bacteria. In spite of the many successful applications, several researchers have reported that bacteria based biofilters exhibit diminished treatment performance and dramatically decreased elimination capacity upon excessive drying or decrease in pH of the biofilter packing media [2, 3, 4]. Recent studies have revealed that some fungal species are not only more tolerant to low moisture content and acidification of the biofilter media but also that they are able to rapidly degrade a wide range of organic chemicals [5, 6].

Biofilter success is obviously dependent on the biodegradability of contaminants [1, 7]. Although many fungal species have been tested for their ability to degrade non-volatile compounds, relatively few accounts of fungal degradation of VOCs appear in the literature. As the initial step in testing a fungal-based biofilter system, five fungal species were screened to test their ability to degrade a variety of VOC contaminants using a novel culturing approach. Experiments were conducted at a range of pH values in order to determine the optimum pH level for fungal growth on the compounds tested.

MATERIALS AND METHODS

Preparation of pure cultured fungi

Five fungal species, *Exophiala lecanii-corni*, *Mucor rouxii* (ATCC 44260), *Phanerochaete chrysosporium* (white rot fungus) (ATCC 24725), *Cladosporium sphaerospermum* (ATCC 200384), and *Cladosporium resinae*(ATCC 34066) were streaked in pure culture on agar petri plates (1.0 g malt extract, 0.5 g yeast extract, and 1.5 g pure (plain) agar in 100 mL of deionized water). All reagents except for agar were obtained from Sigma (St. Louis, MO). Agar was obtained from Fisher Scientific (Pittsburgh, PA). Unless otherwise noted, all media and laboratory instruments contacting the fungi were sterilized by autoclaving at 120 ^{o}C and 15 psi for 30 minutes, and aseptic technique were employed throughout the studies.

The agar petri dishes were incubated at 30 ± 1 ^{o}C for 7 days when appreciable growth was observed for all cultures. Then, 5.0 mL of a sterile nutrient solution prepared by adding the constituents listed in Table 1 to deionized water was added to the dishes grown with fungi. The surface each of dish was gently scraped using a sterile loop, and the dishes were shaken for 30 sec. The resulting fungal suspension was removed from each agar petri dishes and transferred into a centrifuge tube containing 10 mL of fresh nutrient solution. The centrifuge tubes were then vortexed for 2 minutes before being centrifuged for 2 minutes at 3000 g. The supernatant was

decanted, 10 mL of fresh nutrient solution was added, and the rinse was repeated twice. Finally, the fungi were re-suspended in 30 mL of fresh nutrient media.

Table 1. Nutrient solution

Compound	Concentration
NH_4NO_3	1.25 g/L
KH_2PO_4	1.0 g/L
$MgSO_4 \cdot 7H_2O$	0.5 g/Ll
$CaCl \cdot 2H_2O$	0.02 g/L
$CuCl_2 \cdot 2H_2O$	0.17 mg/L
$CoCl_2 \cdot 6H_2O$	0.24 mg/L
$ZnSO_4 \cdot 7H_2O$	0.58 mg/L
$MnSO_4 \cdot H_2O$	1.01 mg/L
$Na_2MoO_4 \cdot 2H_2O$	0.24 mg/L
$NiCl_2 \cdot 6H_2O$	0.10 mg/L
$FeSO_4 \cdot 7H_2O$	1.36 mg/L
Streptomycin sulfate	4.5 mg/L

Inoculation and culture

One milliliter of the fungal suspension was transferred to a glass petri dish (1.5 cm x 10.0 cm) containing 5 ceramic pellets (R-635 Celite®, Lompoc, CA) that were previously heated to 550^{o}C in a muffle furnace to remove organic contaminants. 5.0 mL of nutrient solution prepared as described in Table 1 but subsequently adjusted to an initial pH value of 3, 5, or 7 (using 10% NaOH (w/v) or 1.0 M HCl) prior to autoclaving was also added to each petri dish. After inoculation, the petri dishes were gently shaken to distribute the fungal suspension over the surface of all pellets. Three short segments of sterile Teflon tubing (approximately 0.5 cm in length) cut lengthwise were fixed around the edge of each petri dish in order to support the dish cover and provide a gap of approximately1mm for diffusion of VOCs.

Dishes inoculated with fungi were then placed in an airtight glass desicator that had been sterilized by surface treatment with an ethanol solution (75% ethanol and 25% sterile deionized water (v/v)) and allowed to air dry in a laminar flow hood supplied with hepa filtered air. The glass desicators contained two additional glass petri dishes below the ceramic support plate. One dish contained a 0.85% (w/v) sterile NaCl solution to control relative humidity, and the other dish was used as a surface to evaporate VOC. To add the VOC to the gas headspace inside the desicator, 100 µL of VOC (neat) was added to the evaporation dish prior to sealing. The desicators were then incubated at ambient laboratory temperature (23 ± 2 ^{o}C). Each desicator

was unsealed, and 100 μL of VOC was added every two days for the remainder of the experiment. Each VOC was tested separately. The VOCs tested included benzene (100%, from J.T. Baker, Phillipsburg, NJ), methyl ethyl ketone (99.9% from Fisher Scientific, NJ), n-butyl acetate and p-xylene (99%, from Aldrich Chemical Co. Inc., Milwaukee, WI).

Five fungal species, *Exophiala lecanii-corni*, *Mucor rouxii*, *Phanerochaete chrysosporium*, *Cladosporium sphaerospermum*, and *Cladosporium resinae* were tested with each VOC at three different pH values. Because the inoculation procedure involved addition of 1.0 mL of fungal suspension to 5.0 mL of nutrient solution adjusted to pH 3, 5, or 7, the pH after inoculation was approximately 3.5, 5.0 or 6.5, respectively. Each treatment was repeated in triplicate. Thus, a total of nine dishes were used for each VOC and fungal species combination tested. Time was measured in days from the time of inoculation.

Additionally, two controls without VOC addition were adopted for each pH and fungi combination tested. The first control (arbitrarily named Control 1) consisted of replicate dishes that were prepared and incubated in a desicator exactly as described above except that no carbon source (VOC) was added to the dessicator. This was used to determine if the fungi grew in the absence of VOC constituents (e.g., by utilizing endogenous carbon reserves, degrading streptomycin, etc.). The second control (arbitrarily named Control 2) consisted of replicate dishes that were prepared and incubated in a desicator exactly as described above except that 1.0 mL of nutrient media prepared as described in Table 1 with the addition of 5.0 g/L glucose was added to each dish prior to incubation. This was used to confirm that a viable culture was used in the inoculation.

At two-day intervals, the petri dishes were examined for visible growth of fungi. When heavy visible growth was readily apparent, incubation was stopped and the cultures were recorded as being capable of utilizing the compound as the sole carbon and energy source under the conditions tested. In the cases where no visible growth was observed, experiments were continued for a minimum of 30 days. When no visible growth was observed even after 30 days of incubation, the culture was recorded as not being capable of utilizing the compound as the sole carbon and energy source under the conditions tested.

RESULTS

Exophiala lecanii-corni

The growth performance of *Exophiala lecanii-corni* is summarized in Table 2. No visible growth was observed in any Control 1 petri dishes (no carbon source) during the 30-day incubation period. On the other hand, fungal growth was observed in Control 2 (glucose supplied as carbon source) 2 days after inoculation for pH 3.5 and 5.0, and 4 days after inoculation for pH 6.5.

Visible fungal growth in three VOC treatments, benzene, methyl ethyl ketone, and n-butyl acetate was observed for all three pH conditions starting on Day 4. For methyl ethyl ketone treatments, more fungal biomass was observed at pH values of 3.5 and 5.0 than was observed at pH 6.5. From Day 6 onward, the largest amount of biomass was observed at pH 5.0 for these

three VOC treatments, followed by pH 3.5 and 6.5. Fungal growth was much more abundant with n-butyl acetate and methyl ethyl ketone than with benzene or glucose.

Fungal growth in the p-xylene treatment was much slower to develop compared with cultures supplied with the other three VOCs (benzene, methyl ethyl ketone, and n-butyl acetate). Visible growth was not observed until 12 days of incubation at pH 6.5 and 14 days at pH 5.0 and 3.5. Moreover, the fungal biomass in the p-xylene treatments was far less than that in the other three VOC treatments.

The fungi were observed to grow not only on and around the Celite pellets but also attached to the bottom of the glass petri dishes. Compared with the glucose treatment, the fungi grown in all VOC treatments were more firmly attached to the glass dish.

Table 2. Growth performance of *Exophiala lecanii-corni*

Treatment	pH 3.5	pH 5.0	pH 6.5
Control 1 (no C source)	–[(1)]	–	–
Control 2 (glucose)	***[(2)]	**	*
methyl ethyl ketone	****	*****	***
n-butyl acetate	****	*****	***
p-xylene	*	*	*
benzene	**	***	*

[(1)] no visible growth, [(2)] * represents visible growth, and the more *, the larger fungal biomass

Mucor rouxii

Table 3 summarizes the growth performance of *Mucor rouxii*. No visible growth was observed in any of the Control 1 dishes throughout the duration of the experiment. Visible growth of *Mucor rouxii* was observed in Control 2 (glucose added) on Day 2 for pH 3.5 and 6.5 and on Day 4 for pH 5.0.

Except for the n-butyl acetate where abundant growth occurred from Day 2 onward for all pH values, no visible growth was observed during the 30-day incubation for any of the other VOCs tested. Unlike the fungus *Exophiala lecanii-corni*, *Mucor rouxii* was present around the ceramic pellets and the surface of liquid media in the dishes instead of attaching to the edge of the dish bottom.

Table 3. Growth performance of *Mucor rouxii*

Treatment	pH 3.5	pH 5.0	pH 6.5
Control 1 (no C source)	–[(1)]	–	–
Control 2 (glucose)	**[(2)]	**	**
methyl ethyl ketone	–	–	–
n-butyl acetate	****	*****	******
p-xylene	–	–	–
benzene	–	–	–

[(1)] no visible growth, [(2)] * represents visible growth, and the more *, the larger fungal biomass

Cladosporium resinae

Growth performance of *Cladosporium resinae* is summarized below in Table 4. No visible growth was observed in any of the Control 1 dishes (no carbon source added) during the 30-day incubation period. Visible growth was observed in Control 2 (glucose added) starting on Day 2 for pH 5.0 and on Day 4 for pH 3.5 and 6.5.

Table 4. Growth performance of *Cladosporium resinae*

Treatment	pH 3.5	pH 5.0	pH 6.5
Control 1 (no C source)	–[(1)]	–	–
Control 2 (glucose)	*[(2)]	**	**
methyl ethyl ketone	*	****	****
n-butyl acetate	****	*****	***
p-xylene	–	–	–
benzene	–	–	–

[(1)] no visible growth, [(2)] * represents visible growth, and the more *, the larger fungal biomass

Visible growth in all n-butyl acetate and methyl ethyl ketone treatments was observed starting on Day 4. During the first week, the amount of growth on cultures supplied with methyl ethyl ketone was greatest at pH 6.5 followed by pH 5.0 and then pH 3.5, but from Day 8 onward, the amount of growth at pH 5.0 increased and took the first position of the sequence. The sequence of growth amount for n-butyl acetate treatment was greatest at pH 5.0 followed by pH 3.5 and then pH 6.7 throughout. No visible growth was observed in any benzene or p-xylene treatments during the 30-day incubation period.

When growth was observed, *Cladosporium resinae* was present around the Celite pellets and attached to the dish bottom.

Phanerochaete chrysosporium

The growth performance of *Phanerochaete chrysosporium* is summarized in Table 5. No visible growth was observed in Control 1 cultures (no carbon source added) during the entire incubation period. In Control 2 (glucose added), visible fungal growth began on Day 4 for all pH levels.

From Day 6 onward, visible growth was observed on methyl ethyl ketone treatments at pH 6.5 and 5.0, with more growth observed at pH 6.5 than at pH 5.0. Interestingly, no visible growth was observed on methyl ethyl ketone treatments at pH 3.5 during the incubation period. Growth in the n-butyl acetate treatments at all pH levels was observed starting on Day 4. The highest growth was observed at pH 5.0, followed by pH 6.5 and 3.5. Growth on benzene was observed from Day 8 onward for pH values of 5.0 and 6.5, with more growth observed at pH 5.0 than that at pH 6.5. When growth was present, *Phanerochaete chrysosporium* grew around the ceramic pellets and attached on the dish bottom. No visible growth was observed for benzene treatments at pH 3.5 during the incubation period. No visible growth for cultures supplied with p-xylene was observed at any pH levels during the 30-day incubation.

Table 5. Growth performance of *Phanerochaete chrysosporium*

Treatment	pH 3.5	pH 5.0	pH 6.5
Control 1 (no C source)	–[(1)]	–	–
Control 2 (glucose)	*[(2)]	**	**
methyl ethyl ketone	–	**	***
n-butyl acetate	**	****	***
p-Xylene	–	–	–
Benzene	–	**	*

[(1)] no visible growth, [(2)] * represents visible growth, and the more *, the larger fungal biomass

Cladosporium sphaerospermum

The growth performance of *Cladosporium sphaerospermum* is summarized in Table 6. No growth was observed in any of the Control 1 cultures during the 30-day incubation period. Visible growth in the Control 2 treatments began on Day 2 for all pH levels. *Cladosporium sphaerospermum* was capable of growing on all four VOCs tested; however, the amount of observed growth differed markedly between cultures supplied with different compounds.

Visible growth was observed in n-butyl acetate treatments at all pH levels from Day 2 onward. Growth was observed in methyl ethyl ketone treatments at pH 6.5 and 5.0 beginning on Day 2,

while growth at pH 3.5 was somewhat delayed. Cultures supplied benzene at pH 6.5 and 5.0 produced visible growth beginning on Day 4, while cultures at pH 3.5 took longer to develop. Visible growth for cultures supplied with p-xylene was observed starting at Day 8 for all three pH levels. Biomass growth for cultures supplied with n-butyl acetate was greatest at pH 5.0 followed by pH 3.5 and then pH 6.5. For cultures supplied with either methyl ethyl ketone or benzene, the amount of biomass was greatest at pH 6.5 followed by pH 5.0 and then pH 3.5. For cultures supplied with p-xylene the amount of biomass was greatest at pH 5.0 followed by pH 6.5 and then pH 3.5.

Abundant fungal growth was observed on both n-butyl acetate and methyl ethyl ketone. *Cladosporium sphaerospermum* was present around the celite pellets and on the surface of the liquid media in dishes.

Table 6. Growth performance of *Cladosporium sphaerospermum*

Treatment	pH 3.5	pH 5.0	pH 6.5
Control 1 (no C source)	–[(1)]	–	–
Control 2 (glucose)	**[(2)]	**	*
Methyl ethyl ketone	*	***	****
n-butyl acetate	*****	****	******
p-Xylene	*	**	*
Benzene	*	**	**

[(1)] no visible growth, [(2)] * represents visible growth, and the more *, the larger fungal biomass

DISCUSSION

For all of the fungal species tested, no visible growth was observed in any of the Control 1 petri dishes in which no glucose or VOC was added. On the other hand, visible growth was observed in all of the Control 2 petri dishes where glucose but no VOC was supplied. These results indicate that a viable fungal culture was inoculated into each of the treatments and that the inoculated culture was not capable of producing visible growth in the absence of VOC supply. Thus, any visible growth observed on petri dishes where fungal inocula were supplied with VOC was taken to be a direct indication of growth on that VOC as a sole carbon and energy source.

A summary of fungal ability to utilize the VOCs under the conditions tested in this study appears in Table 7. Results indicate that the fungi *Exophiala lecanii-corni* and *Cladosporium sphaerospermum* can effectively utilize all four of the compounds tested (n-butyl acetate, methyl ethyl ketone, benzene, and p-xylene) which are commonly found in paint spray booth off-gases. These results suggest that fungi *Exophiala lecanii-corni* and *Cladosporium sphaerospermum* might be the promising candidates for use in fungal-based biofilters to treat gas streams contaminated by wide range of VOCs.

Table 7. Growth performance of fungi growing on VOCs

	Exophiala lecanii-corni	**Cladosporium sphaerospermum**	**Phanerochaete chrysosporium**	**Cladosporium resinae**	**Mucor rouxii**
No carbon source	–[(1)]	–	–	–	–
Glucose	+[(2)]	+	+	+	+
n-Butyl acetate	+	+	+	+	+
Methyl ethyl ketone	+	+	+	+	–
Benzene	+	+	+	–	–
p-Xylene	+	+	–	–	–

[(1)] - indicates no visible growth was observed, [(2)] + indicates visible growth was observed

To our knowledge, the capability of *Exophiala lecanii-corni* to grow with benzene, p-xylene, or methyl ethyl ketone as a sole carbon and energy source has not been reported previously. Moreover, in apparent contrast to our results, Woertz and Kinney (2000) recently reported that the same strain of *Exophiala lecanii-corni* was not able to degrade benzene or p-xylene in liquid cultures [8]. These apparently contradictory results are likely due to differences in the culturing techniques utilized. Woertz and Kinney conducted their experiments by placing *Exophiala lecanii-corni* cultures in liquid suspensions that were agitated by placing them on a shaker table during incubation. Studies described herein used static, attached growth culturing techniques. Previous studies on other fungal species have concluded that agitation as well as attached versus suspended growth conditions can markedly affect the morphology and metabolism of fungi. Additionally, studies described herein supplied the fungal culture with both ammonia and nitrate as a nitrogen source rather than only ammonia-nitrogen, and differences in the nutrient supply might have led to differences in metabolism.

It has been previously reported that *Exophiala jeanselmei*, a fungus closely related to *Exophiala lecanii-corni*, was able to assimilate a class of benzene-like compounds including 4-ethylphenol [9], styrene [10], and toluene [11]. The ability of *Exophiala lecanii-corni* to use n-butyl acetate and methyl ethyl ketone as a sole carbon and energy source under the conditions of this study are consistent with other researchers who have reported that *Exophiala lecanii-corni* can degrade n-butyl acetate, methyl propyl ketone, and ethyl ethoxypropionate in agitated suspended-growth conditions [8].

Maximum growth of *Exophiala lecanii-corni* was observed at a pH value of 5.0 while exposed to all VOCs tested except for p-xylene where maximum growth was observed at pH 6.5. It is interesting to note, however, that highest growth rates were obtained at pH 3.5 when glucose was supplied as the sole carbon and energy source. Most microorganisms grow most rapidly when cultured at a specific pH range, and changes in the pH of biofilter media can strongly influence microbial activity. Optimal pH conditions for compost biofilters have been reported to be between 7 and 8, a range preferred by bacteria and actinomycete [1]. Fungal-based filters have been reported to be more tolerant to low pH values [16]. In this study, the optimal pH for most of VOCs treatments was around 5.0, and an optimal pH of 3.5 for most of fungi tested with glucose as sole carbon and energy source, suggesting that the optimal value of pH might change with variation in type of carbon source.

To our knowledge, the capability of *Cladosporium sphaerospermum* to grow with benzene, p-xylene, or methyl ethyl ketone as sole carbon and energy sources has not been reported previously. One previous study reported that *Cladosporium sphaerospermum* is capable of degrading toluene as a sole carbon and energy source when grown suspended in liquid media [11]. Other previous studies on *Cladosporium sphaerospermum* demonstrated that this fungi can use toluene, styrene, ethylbenzene, propylbenzene, and some other benzene compounds as sole carbon and energy sources; however, no growth of *Cladosporium sphaerospermum* with *o*-xylene, benzene, or phenol was found in their study[12]. The apparently conflicting results between this study and that previously reported are likely due to the difference in culture techniques. In the study conducted by Weber and his colleagues, the fungi were grown in liquid media containing a phosphate buffer at pH 7.0. In addition to differences resulting from suspended growth versus attached growth process and differences in composition of nutrient solutions, results described herein indicate that higher growth rates are observed for more acidic pH levels than were utilized in the previous study. Lower pH levels may have been necessary for growth on these compounds.

There have been few accounts of VOC degradation by *Mucor rouxii* in the literature. Results of this study indicate that *Mucor rouxii* is capable of using n-butyl acetate as a sole carbon and energy source; however, the fungi was unable to utilize methyl ethyl ketone, benzene, or p-xylene under the conditions tested.

Other than early reports that *Cladosporium resinae* could grow when supplied with jet fuel, there are few accounts in the literature of VOC degradation by *Cladosporium resinae*. Of the four compounds tested in these studies, *Cladosporium resinae* could only use the organic acid (n-butyl acetate) and ketone (methyl ethyl ketone) as sole carbon and energy sources. Although this fungi exhibited less biomass growth and an apparently lower growth rate than the other fungi tested, because the amount of the inoculum was not standardized during the screening process and specific growth rates and specific contaminant removal rates have not yet been determined, it is not certain whether the contaminant removal rates were different.

Numerous studies have described that the white-rot basidiomycetous fungus *Phanerochaete chrysosporium* is capable of effectively degrading polynuclear aromatic hydrocarbons (PAHs) and other recalcitrant organic compounds [13, 14, 15]. Results from this study indicate that *Phanerochaete chrysosporium* is capable of degrading n-butyl acetate, methyl ethyl ketone, and benzene. *Phanerochaete chrysosporium* could not degrade p-xylene under the conditions

imposed by this study, which is the same as the result from Braun-Lüllemann *et al*. (1995) by straw culture test [5].

CONCLUSIONS

The experimental protocol utilized in the studies described herein is a useful tool for assessing the ability of different fungal species to degrade gas-phase VOCs. This method may serve as a better predictive tool for characterizing the ability of microorganisms to degrade compounds under the conditions expected in biofilters where attached growth processes.

Several fungal species are capable of degrading VOC constituents commonly found in paint spray booth off-gases. In particular, n-butyl acetate, methyl ethyl ketone, benzene, and p-xylene can all be used as the sole carbon and energy source by some fungal species. *Exophiala lecanii-corni* and *Cladosporium sphaerospermum*, which both degraded all four of the compounds tested, are promising candidates for use in fungal biofilters to treat gas streams contaminated by a variety of VOCs. Further studies are underway to determine the ability of these fungi to degrade additional VOCs and mixtures of VOCs. Additional studies are underway to compare the performance of biofilters inoculated with *Exophiala lecanii-corni* and *Cladosporium sphaerospermum* with those inoculated with undefined mixed cultures of bacteria.

ACKNOWLEDGEMENTS

The authors would like to gratefully acknowledge the Louisiana Board of Regents and the Strategic Environmental Research and Development Program (SERDP) for financial support of this research.

REFERENCES

1. Leson, G.; Winer, A.M. (1991) Biofiltration: An Innovative Air Pollution Control Technology for VOC Emissions. *J. Air Waste Manage. Assoc.*, 41, 1045-1054.

2. Agathos, S.N.; Hellin, E.; Ali-Khodja, H.; Deseveaux, S.; Vandermesse, F.; Naveau, H. (1997) Gas-phase Methyl ethyl ketone Biodegradation in a Tubular Biofilm Reactor: Microbiological and Bioprocess Aspects. *Biodegradation*, 8, 251-264.

3. Auria, R.; Aycaguer, A.C.; Devinny, J.S. (1998) Influence of Water Content on Degradation Rates for Ethanol in Biofiltration, *Journal of the Air & Waste Management Association*, 48, 65-70.

4. Severin, B.F.; Shi, J.; Cybul, B.G.; Neilson, L.M.; Furstenberg, J.L. (1995) High-Rate Low Volume Biofilters for Industry, Water Environment Federation; Industrial Wastes Technical Conference; Multimedia Pollution Control and Preventions, Pittsburgh, PA.

5. Braun-Lüllemann, A.; Johannes, C.; Majcherczyk, A.; Hüttermann (1995) The Use of White-Rot Fungi as Active Biofilters. In Heichee, RE, Sayles, RD, and Skeen, RS (Eds.), *Biological Unit Processes for Hazardous Waste Treatment*, 3, 235-240. Battelle Press.

6. Woertz, J.; Kinney, K.A.; McIntosh, P.; Szaniszlo, P. (1999) Toluene Removal in a Fungal Vapor Phase Bioreactor. *Proc. of the Fifth International Symposium on In-Situ and On-Site Bioremediation*, San Diego, CA, April 19-22, 1999.

7. Medina, V.F.; Webster, T.; Ramaratnam, M.; and Devinny, J. (1995) Treatment of Gasoline Residuals by Granular Activated Carbon Based Biological Filtration. *J. Environment. Science and Health*, A30, 407-422.

8. Woertz, J.; Kinney, K.A. (2000) Use of Fungus *Exophiala Lecanii-corni* to Degrade a Mixture of VOCs. *Proc. 2000 USC-TRG Conference on Biofiltration*, Los Angeles, CA, Oct. 19-20, 2000, 151-158.

9. Middelhoven, W.J. (1993) Catabolism of Benzene Compounds by Ascomycetous and Basidiomycetous Yeasts and Yeast-like Fungi. *Antonie van Leeuwenhoek*, 63, 125-144.

10. Cox, H.H.J.; Magielsen, F.J.; Doddema, H.J. (1996) Influence of the Water Content and Water Activity on Styrene Degradation by *Exophiala jeanselmei* in Biofilters. *Appl Microbiol Biotechnol*., 45, 851-856.

11. Sanchez-Pena, S.R.; Kazenski, S.L.; Woertz, J.L.; Kinney, K.A.; Szaniszlo, P.J. (2000) An Assessment of Fungal Biofiltration of Volatile Organic Compounds. *Proc. of the AWMA Conference*, Salt Lake City, UT, 2000.

12. Weber, F.L.; Hage, K.C.; de Bont, J.A.M. (1995) Growth of the Fungus *Cladosporium sphaerospermum* with Toluene as the Sole Carbon and Energy Source. *Appl Environ Microbiol*, 61, 3562-3566.

13. Wariishi,H.; Valli, K.; Gold, M.H. (1991) *In vitro* Depolymerization of Lignin by Manganese Peroxidase of *Phanerochaete chrysosporium*. *Biochem. Biophys. Res. Commun*. 176, 269-275.

14. Haught, R.C.; Neogy, R.; Vonderhaar, S.S.; Krishnan, E.R.; Safferman, S.I.; Ryan, J. (1995) Land Treatment Alternatives for Bioremediating Wood Preserving Wastes. *Hazardous Waste & Hazardous Materials*, 12, 329-343.

15. Aitken, M.D.; Irvine, R.L. (1990) Characterization of Reactions Catalyzed by Manganese Peroxidase from *Phanerochaete chrysosporium*. *Arch. Biochem. Biophys*., 276, 405.

16. Groenestijn, J.V.; Harkes, M.; Cox, H.; Doddema, H. (1995) Ceramic Materials in Biofiltration. *Proc. of the Conference on Biofiltration*, Los Angeles, California, October 5-6, 1995.